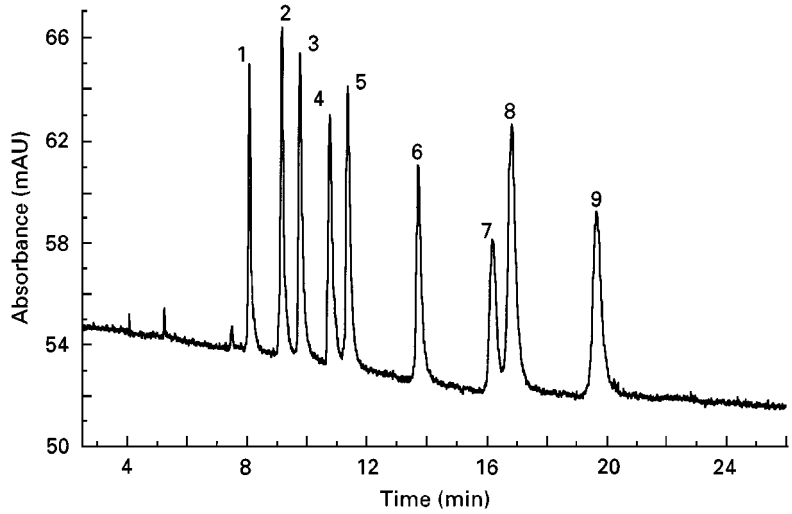

Figure 7 CZE separation of a peptide mixture. Capillary: ethylene/vinyl acetate dynamically coated with polyvinyl alcohol 75 μm i.d., 25/45 cm, buffer: phosphate 50 mmol L^{-1}; pH = 2.5; E = 155 V cm^{-1}, 200 nm; injection 50 mbar, 5 s. 1, Bradykinin; 2, angiotensin II; 3, α-MSH; 4, TRH; 5, LH-RH; 6, leucin enkephalin; 7, bombesin; 8, methionin; 9, oxytocin.

acids and peptides due to the manifold separation modes that can be applied. Short analysis times, easy manipulation of separation conditions and small injection volumes (nanolitres) are further advantages.

The field of biomedical and clinical amino acid and peptide analysis is still under investigation, especially as the transfer and adaptation of the separation modes to a broader range of real samples has to be established. Thus monitoring of *in vivo* processes, e.g. analysis of neurotransmitters in cerebrospinal fluid after online microdialysis, could be realized.

This is directly related to further improvements in reproducibility and detection strategies.

The most promising techniques that will fulfil the demands of trace analysis in biological fluids are CE-LIF and CE-MS.

Future trends are micro-fabricated CE devices implementing CE technology on a microchip and multiple capillary arrays allowing simultaneous analysis of up to 96 samples. Thus, a down-scaling of the analytical process and the performance of high throughput analysis could be achieved.

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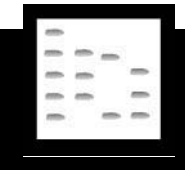
ANAESTHETIC MIXTURES: GAS CHROMATOGRAPHY



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Introduction

Today, anaesthetists normally use mixtures of nitrous oxide and oxygen as a background anaesthetic and carrier to introduce a potent volatile liquid

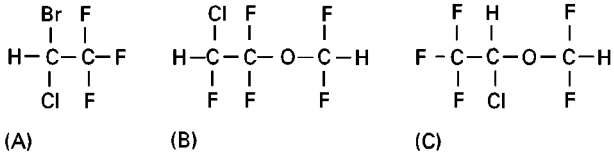

Figure 1 Molecular structures for the volatile liquid anaesthetics (A) halothane, (B) enflurane and (C) isoflurane.

anaesthetic such as halothane (2-bromo-2-chloro-1,1,1-trifluoroethane), isoflurane (1-chloro-2,2,2trifluoroethyl difluoromethyl ether) or enflurane (2-chloro-1,1,2-trifluoroethyl difluoromethyl ether: **Figure 1**) to produce a state of anaesthesia and analgesia and to sedate a patient. Monitoring the patient's inhaled and exhaled breath during surgery is very important as a measure of the anaesthetic uptake and the depth of the anaesthesia. In operating theatres, therefore, physical methods of analysis (e.g. dedicated infrared analysers) are employed on account of their speed of response and continuous display facilities, though most can reliably handle only one component at a time. However, there is still the need to analyse such mixtures for all the major components, either in the course of research programmes involving different agents and different combinations such as inhaled or exhaled mixture analysis, blood and body fluid analysis, anaesthetic pollution studies, thermal decomposition studies or as a back-up to confirm the performance of the dedicated analysers. The major gases present in such mixtures, in addition to air, are carbon dioxide, nitrous oxide and halothane, isoflurane or enflurane (or cyclopropane, which is still in use in some places). If all the components (gases and vapours) need to be detected, gas chromatography is extremely powerful in the separation and quantification of the components, in comparison with the other techniques available.

Instrument Requirements and Procedures

There is no rigid boundary separating the basic instrumental requirements for conventional gas analysis and anaesthetic mixture analysis by gas chromatography. All the theoretical and practical knowledge and basic equipment of conventional gas analysis applies to anaesthetic mixtures and this simplifies the practice of the technique in this specialized field. A dual-column gas chromatograph fitted with a gas sampling valve (operated at room temperature), and equipped with a thermal conductivity detector (TCD) or preferably both TCD and flame ionization detector (FID) is most suitable for all the anaesthetic gas mixture analysis encountered. If a septum-type inlet system is also present, it should be placed next to the gas switching valve.

Sample Handling and Injection

Sample handling and injection techniques are greatly influenced by the source of the analysed samples such as liquid samples containing anaesthetics (e.g. blood, urine, sperm, tissue), low concentration gas samples (e.g. anaesthetics in pollution studies) and high concentration gas samples (e.g. inhaled and exhaled gas mixtures).

Direct injection of a liquid sample to the chromatographic column is very simple, but it is a rather crude approach and has serious disadvantages such as contamination of the sample port, column and detector, alterations in the baseline characteristics and interference by water vapour. The problems associated with the presence of the liquid in the chromatographic system are avoided by the technique of headspace analysis, whereby the vapour above the sample is injected under controlled conditions. Headspace sampling is rapid and is suitable for direct determination of the partial pressure of anaesthetics in blood.

Low concentration samples of liquid anaesthetics trapped in an adsorbent-filled cartridge (integrated sampling or passive dosimeter) in pollution studies are introduced into a gas chromatographic system via a gas sampling valve. Trapped anaesthetics are desorbed from the adsorption cartridge and transferred by the carrier gas to the main chromatographic column by heating the adsorption cartridge rapidly.

Low concentration (spot sampling) and high concentration samples in the gaseous state may be introduced to a gas chromatographic system by a gas-tight syringe (0.1–5.0 mL) with the usual septum-type inlet system. However, this is not a reproducible sample introduction method and creates problems of reliability where quantification of the components is needed. In addition to this, polymeric material such as rubber (e.g. on the barrel of a disposable syringe), plastics, and even glass itself adsorb liquid anaesthetics ($\sim$1–3%) on the contact surface. Adsorption on glass surfaces becomes more important when dealing with mixtures at lower concentrations (**Figure 2**). Therefore, syringe injection should be avoided in quantitative studies.

If gas samples are to be taken repeatedly to produce reproducible quantitative data, a gas sampling valve fitted with the desired size of sampling loop (0.25–10 mL) should be used at a constant temperature and filling pressure (usually ambient). It should be noted that, when using a concentration-sensitive detector such as TCD, the sample size and column diameter relationship must be taken into consideration to avoid column overloading. Several commercial gas sampling valves are available in various configurations. Some operate on the slider with the

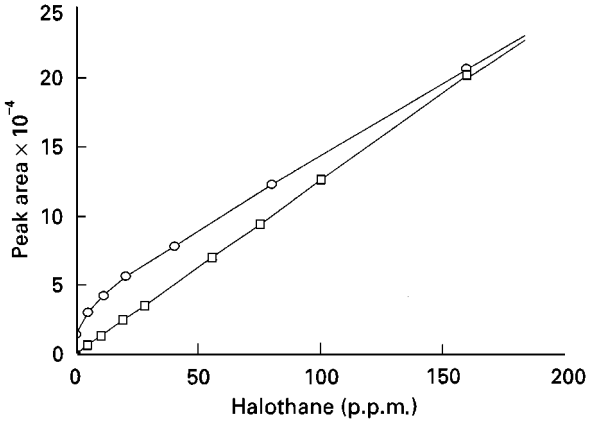

Figure 2 Adsorption of halothane on glass surface at lower concentrations. Squares, cylinder preparation; circles, syringe dilution.

O-ring principle, while others operate by rotation of a Teflon® (polytetrafluoroethylene) or polyimide rotor in various flow paths. The analyst should be aware that some polymeric materials (e.g. silicone rubber O-rings) adsorb anaesthetic vapours to some extent (halothane > isoflurane > enflurane). Gas switching valves made of a stainless-steel body and Teflon® rotor or O-rings are the most suitable choice for anaesthetic purposes. It is important to note that gas sampling valves must not be used with flow control of the carrier gas, as this restricts the filling rate and hence the rate of flushing of the loop, resulting in tailing peaks, Pressure control is used instead.

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Choice of Column

The column has an essential role in the separation process. Optimization of the separation process by suitable choice of chromatographic column, therefore, is the main starting point of any gas chromatographic analysis. Selection of a column is often made on the basis of the nature of the samples and the number of components to be analysed.

Capillary columns have been little used, and mainly for liquid anaesthetic analysis without gas components. The reason for this is the unfavourable retention factors of low boiling compounds on capillary columns operated at room temperature.

Packed columns may be subdivided as liquid partition and solid adsorbent columns. Almost all the anaesthetic gas analysis reported so far has been performed on packed columns of various lengths, either single or combined, commonly with 1/8 in and 1/4 in o.d. Liquid partition columns are generally employed to separate the high boiling or heavier components such as liquid anaesthetics, while solid absorbent columns are used for the permanent gases (CO_2, O_2 and N_2).

Synthetic porous polymer beads, which have been in widespread use as solid adsorbent packing material, are available commercially under a variety of trade names (Chromosorb Century Series, Porapak). Columns packed with porous polymer beads are more versatile and capable of separating each of the individual groups of components such as light gases and liquid anaesthetics at different temperatures as well as their complex mixtures with suitable temperature and column arrangements. No special treatment is required to obtain symmetrical peaks as they are chemically inert to the anaesthetic substances under the chromatographic conditions employed (usually 20–220°C). The combined effects of increasing viscosity of the carrier gas and expansion of the stationary phase as the temperature rises result in a very marked decrease in the carrier flow (**Figure 3**), e.g. a temperature rise from ambient to 200°C decreases the flow of the carrier from around 50 mL min^{-1} to 20 mL min^{-1} at 40 psig (2.7 bar) He inlet pressure, with a 2 m, 80–100 mesh Chromosorb 101 column. Nevertheless, the chromatography remains adequate and gives peaks for the liquid anaesthetics which are easily integrated. The size of the particles, expressed in mesh size, is very important in the column efficiency as the separation is provided by the surface and structure characteristics of the packing material. When the size of the particles is reduced, the column efficiency is increased and so is the inlet pressure because of the high pressure resistance of the column. At the present time, 80/100 mesh is the most widely used fraction; however, in instances where higher efficiency is needed, 100/120 mesh is frequently used.

Column Tubing Materials

Since most anaesthetic mixtures contain at least one volatile liquid component other than the permanent

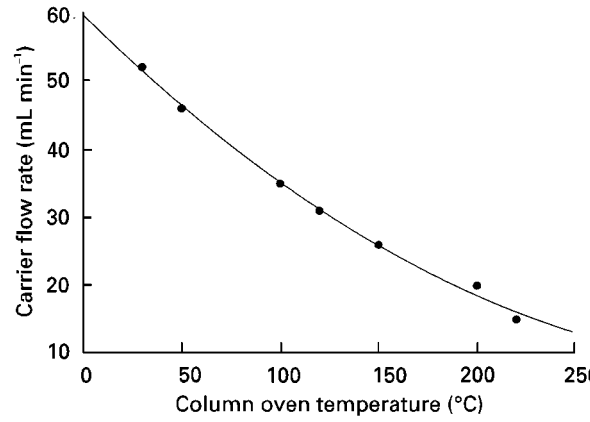

Figure 3 Relationship of temperature to flow rate of a porous polymer packing (80/100 mesh). Flow rate = $3.86 \times 10^{-4} T^2 - 0.283 T + 59.6$.

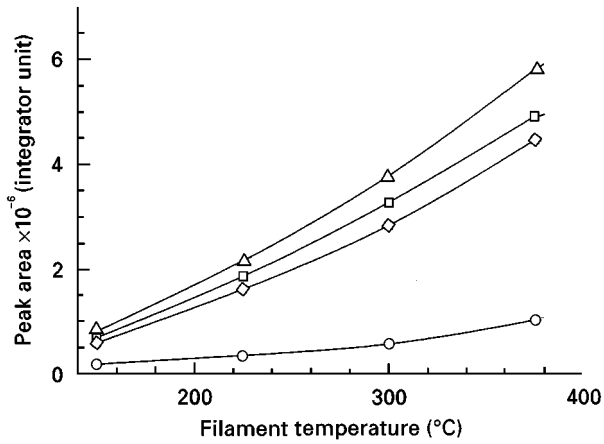

Figure 4 Variation of the hot-wire TCD responses with detector filament temperature. Circles, halothane; squares, nitrous oxide; triangles, carbon dioxide; diamonds, air.

gases, operating temperatures with solid adsorbent columns are considerably higher (e.g. 150–220°C) than those required for the separation of the permanent gas alone. Therefore, many of the commonly used tubing materials for permanent gas analysis at lower temperatures may not safely be used in anaesthetic gas analysis. For example, anaesthetic vapours (particularly halothane) tend to decompose in contact with metals (or metal/metal oxide) such as aluminium ($\sim 200°C$) and copper ($> 250°C$) at elevated temperatures, producing a number of halogenated products. Relatively inert materials such as glass and stainless steel may safely be used as column tubing materials for anaesthetic separation purposes at high operating temperatures. Since mixtures contain large amounts of oxygen, heated septum-type injection ports should have a glass liner to prevent metal–liquid anaesthetic contact at higher temperature settings.

Choice of Detectors

The most commonly used detectors in anaesthetic gas analysis are TCD, FID and electron capture detector (ECD).

TCD is concentration-sensitive and has been the most widely used in chromatographic analysis for the determination of gases, and for any applications in explosion hazard areas. If inorganic gases, besides

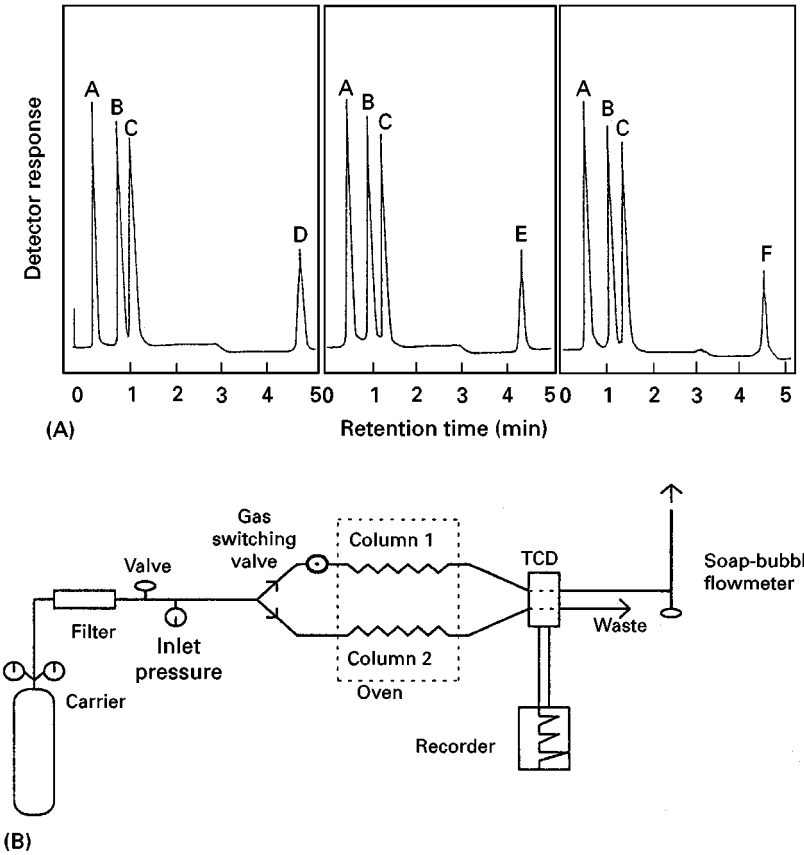

Figure 5 (A) Gas chromatograms for the single-column separation of anaesthetics by temperature programming (linear or nonlinear). A, Air; B, carbon dioxide; C, nitrous oxide; D, halothane; E, isoflurane; F, enflurane. (B) Simple set-up of a temperature-programmed (linear or nonlinear) dual-column chromatograph.

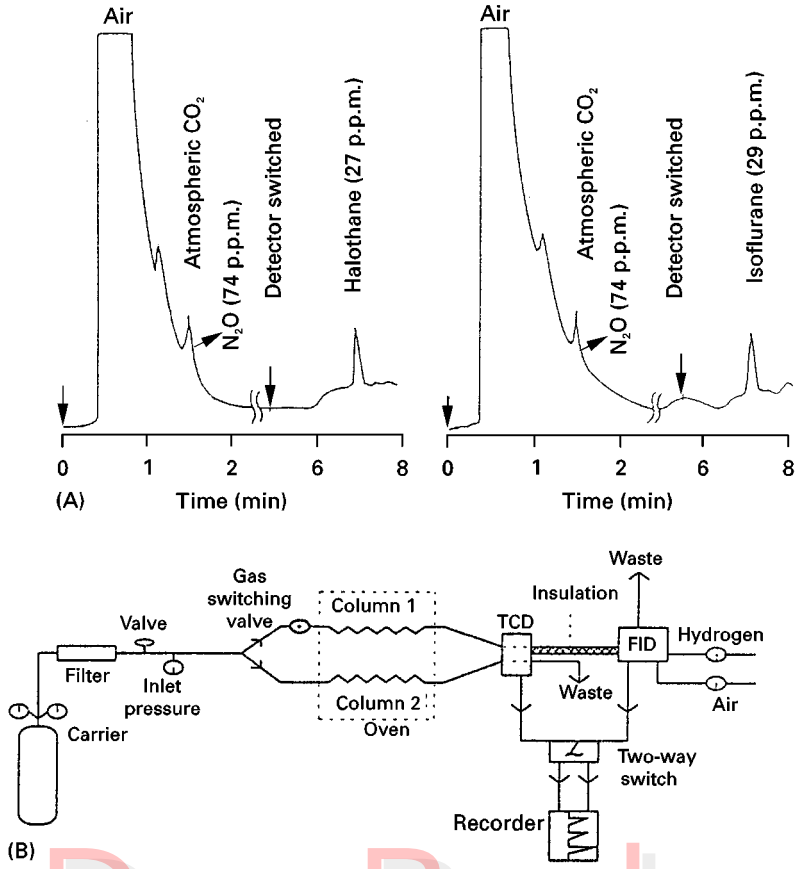

Figure 6 Chromatograms for dual detector chromatography (A) halothane (left) and isoflurane (right) in atmospheric air. (B) Simple set-up of a dual detector chromatograph.

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liquid anaesthetics, need to be analysed, TCD is the detector of choice due to its universal response to almost all substances and its very large linear dynamic range. Because of its relatively poor sensitivity, it is unsuitable for the determination of low concentrations (<40 p.p.m.) without employing extreme detector conditions and large sample volumes. The nondestructive character of the TCD enables it to be used in dual-column chromatography by utilizing two channels simultaneously or in series with another detector such as the FID. Sensitivity of the hot-wire TCD depends on the temperature difference between filament and cell wall temperature (**Figure 4**), and higher chromatographic responses are obtained at higher filament temperatures.

The ECD is very sensitive to electrophilic species such as polyhalogenated anaesthetics and also to nitrous oxide, but its linear dynamic range is limited to a range of about 10^4 and it can easily be saturated. For this reason, it is generally employed for the low concentration determination of liquid anaesthetics and nitrous oxide. Since oxygen and water influence the detector sensitivity, these compounds must be rigorously removed from the carrier and make-up gases. Contamination also causes serious interference. The detector must be held at an elevated temperature, always with a steady flow of carrier gas, and must be regularly baked out to ensure cleanliness. All these factors make ECD a difficult detector in anaesthetic gas analysis.

The very widely used FID is a mass-sensitive detector, with the disadvantage compared to the TCD that it is destructive. It responds to virtually all organic components but does not respond to the permanent gases. In the great majority of studies where only the determination of the volatile liquid anaesthetics is needed (e.g. blood and body fluid analysis), FID is used. If the analysis includes nitrous oxide in addition to liquid anaesthetics, the ECD alone may be chosen. For low concentration analysis, TCD and FID may be connected in series to determine the permanent gases and the liquid anaesthetics.

Choice of Carrier Gas

Choice of the carrier gas depends on the detector employed. For FID and ECD, carrier gas is not critical

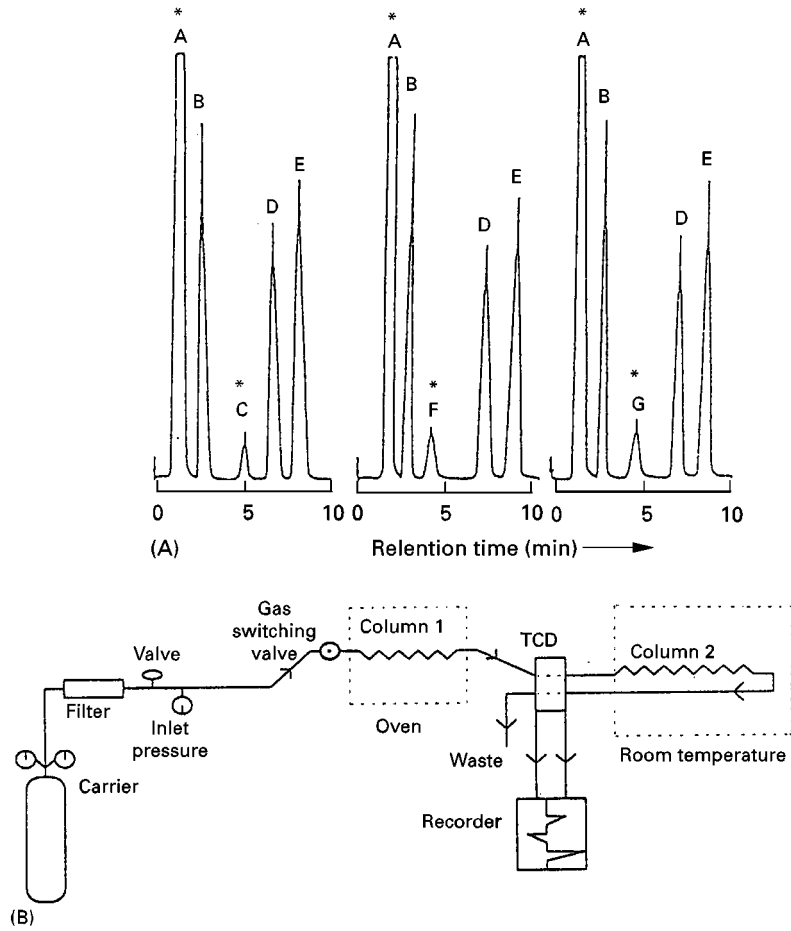

Figure 7 (A) Gas chromatograms for the dual-column separation of A, combined peak; B, air; C, halothane; D, carbon dioxide; E, nitrous oxide; F, isoflurane; G, enflurane; *, converted peaks. (B) Simple set-up of a temperature-programmed (linear or nonlinear) single-column chromatography.

and nitrogen may be used for most chromatographic purposes in anaesthetic analysis. For the operation of the TCD, hydrogen and helium give the highest sensitivities, but helium is preferred on safety grounds.

Tactics for the Anaesthetic Gas Analysis

It is usually required to measure a number of the components in an anaesthetic mixture (e.g. vapours and permanent gases), and a single column in a single isothermal run rarely meets this need. Although isothermal operation is preferred whenever possible, temperature programming may be used to improve the separation process. The magnitude of the temperature range depends on the sample components and the nature of the column packing materials. The disadvantage of temperature programming is that time is required at the end of an analysis to return the initial column temperature.

Using temperature as a variable is not, however, the only approach. Improved separations can be achieved by employing mixed column packing materials in various proportions and column lengths (e.g. porous polymers and molecular sieves) and multicolumn (parallel or serially) arrangements operating in tandem or at different temperatures with single or multidetector systems. Utilizing these approaches in various multicolumn and detector combinations allows the analyst to separate most mixtures of anaesthetics and permanent gases. **Figures 5–7** show the various arrangements with examples of the chromatograms obtained.

Quantitative Analysis

To be able to carry out quantitative work, the gas chromatograph must be calibrated with accurately prepared mixtures of known composition. Dynamic methods for calibration such as gas stream

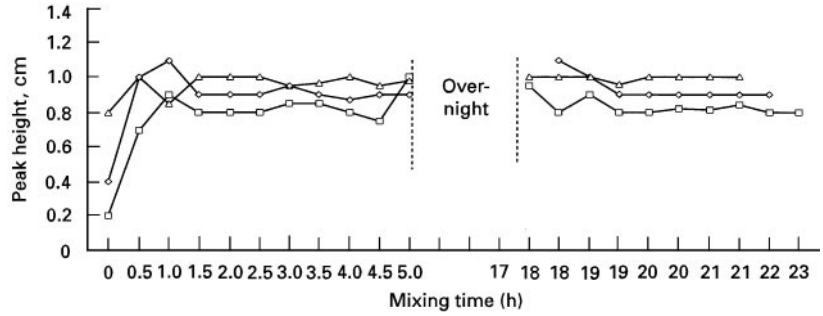

Figure 8 Mixing time for halothane prepared in helium. Squares, 1.1% halothane at 8.5 bar; diamonds, 1.2% halothane at 5.0 bar; triangles, 1.4% halothane at 3.1 bar.

mixing, permeation, diffusion and evaporation generate continuous flows of mixtures of known composition and are generally employed in studies where large volumes of standards at low concentrations are needed. Static methods for producing standard gas mixtures are appropriate when relatively small volumes of mixtures are required at moderately high concentration levels and have been widely used in calibrating gas chromatographic instruments. The preparation of calibration mixtures in gas cylinders involves either volumetric or gravimetric mixing. Gravimetric methods in which the the concentrations are determined from the mass of each component present in the cylinders irrespective of the temperature and pressure of the mixture represent the nearest approach to an absolute method, provided the mixture is homogenous. The mixing rate is inversely proportional to the total pressure and is rapid if thermal or mechanical agitation of some kind is introduced to cause turbulence in the gas (usually the cylinder is rolled in a horizontal position). Without mechanical mixing, equilibration is likely to take several days (**Figure 8**). Syringe dilution methods (even with all-glass syringes) are not suitable for calibration purposes, particularly at lower concentrations, due to the adsorption of the liquid anaesthetics (see Figure 2).

Quantitative evaluation may be performed either by peak height or by peak area. The most commonly used method is based on direct calibration with standard samples which bracket the anticipated values in the unknown sample. The correlation peak value versus concentration generally exhibits a linear plot. The basic condition for successful quantitative analysis is a high degree of constancy of operating conditions and the accuracy of the analysis is significantly affected by apparatus parameters, characteristics of the detector and the skill of the analyst.

Conclusions

It may be concluded that there is no lack of knowledge, equipment and method to perform gas chromatographic separation and quantitative evaluation of all types of anaesthetic mixtures from one to multicomponent mixtures (including light gases and gaseous anaesthetics) in this extensively described well-established field. Nevertheless, the time required for analysis means that gas chromatography is mainly used for anaesthetic research purposes. Separations taking 5–10 min are not acceptable to medical personnel who would require a time scale an order of magnitude less for analysis of patient's breath in an operating theatre. However, there is room for future improvements to simplify the column systems, developing fast and continuous methods with automated samplers to be able to monitor anaesthetic concentrations during surgery.

See also: **II/Chromatography: Gas:** Gas-solid Gas Chromatography; Headspace Gas Chromatography; Detectors: General (Flame Ionization Detectors and Thermal Conductivity Detectors); Detectors: Mass Spectrometry; Detectors: Selective; Sampling Systems. **III/Gas Analysis: Gas Chromatography.**

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